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Short communication

The effects of low-dose ultraviolet light-C treatment on polygalacturonase activity, delay ripening and Rhizopus soft rot development of tomatoes

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Abstract

Low-dose application of hormetic (adj. of hormetin, the agent of hormesis e.g. UV-C) ultraviolet light-C (UV-C) to 'Better Boy' and 'Floradade' tomatoes at maturity resulted in fruits that were significantly, firmer than non-irradiated control fruits at the same stage of maturity. As, firmness increased following UV-C treatment, polygalacturonase (PG) activity decreased. The enzyme activity was lower for UV-C treated fruits than the control. The PG activity in crude extract from decayed tomato tissue infected with *Rhizopons stolonifer* was lower than non-decayed fruits. UV-C treated tomatoes showed a 40% reduction in PG activity in decayed tissue compared to the control at 72 h after treatment. The lesion diameter, and percent infection of non-treated and UV-C treated tomatoes at 72 h after treatment, was 13.4 mm (100% infection), and 5.3 mm (47% infection), respectively.

Keywords: Tomato; Hormesis; Induced resistance; Rhizopus soft rot

1. Introduction

Rhizopus soft rot caused by *Rhizopus stolonifer* (Ehrenb. ex Fr.) is one of the most destructive postharvest storage diseases affecting tomato fruit (*Lycopersicon esculentum* Mill.) (Snowdon, 1992). A relatively new crop protection technology that involved exposing fruits and vegetables low to dose ultraviolet light (UV-C, 254 nm) was first shown by Stevens and colleagues to induce resistance to postharvest storage rots (Stevens et al., 1996; Wilson et al., 1994). Luckey (1980) reported the results of induced resistance of plants to crop diseases by the application of a low or sub-lethal dose(s) of UV-C light. This was in contrast to the germicidal effects of UV-C light, which involves sterilization of the fruit surfaces (Stevens et al., 1998). He termed this phenomenon radiation hormesis, and hormetin is the agent (e.g. hormetic dose of UV-C light) (Luckey, 1980). Liu et al. (1993) reported that the application of a low-dose UV-C of 3.6 KJ/m^{-2}, induced resistance to Rhizopus soft rot of tomatoes. Also, they reported that UV-C improved shelf-life, delayed ripening, and improved fruit quality of tomatoes (Liu et al., 1993; Stevens et al., 1998).

The objectives of this study were: (1) to determine the effects and relationship of low-dose hormetic UV-C exposure on polygalacturonase (PG) activity of tissue extract, and firmness of tomato fruits, and (2) to determine PG activity of decayed tissue extract of UV-C treated and untreated tomatoes inoculated with *R. stolonifer*, the casual agent of Rhizopus soft rot.

2. Materials and methods

2.1. Tomato cultivars

All studies were conducted with 'Better Boy' and 'Floradade' tomato varieties. All fruits used in these

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experiments were similar in size, and free of decay. Tomatoes were harvested at the mature green stage as described by Rick (1987) then exposed to UV-C, and after storage for 4 days at room temperature in the dark (24–27°C and 69% RH) they were sorted into three maturity classifications based on exocarp color according to Rick (1987). The maturity stages used were mature green (stage 1), breaker (stage 2) and orange color (stage 3).

2.2. Fungal inoculum preparation and artificial inoculation

R. stolonifer cultures was maintained in vitro on potato dextrose agar (PDA) in petri dishes at 28°C for 7–14 days. Fruits were surface-sterilized with 95% ethanol, and two wounds per fruit were made with a sterile dissecting needle to a depth of 3 mm. Twenty microlitre of a *R. stolonifer* spore suspension (10^5 spores/ml) was applied to each wound as described previously (Liu et al., 1993; Stevens et al., 1998).

2.3. UV-C irradiation method

A low-pressure mercury-vapor discharge lamp (General Electric, Fairfield, CT) emitting quasi-monochromatic UV radiation at 254 nm was used. Fruits were placed on a tray approximately 10 cm from the surface of the lamp, and exposed to a UV-C dose of 3.6 kJ/m^2, measured by UVX radiometer (UVP, Inc., San Gabriel, CA) for 5 min according to the procedure described by Stevens et al. (1998).

2.4. Polygalacturonse extraction and assay

Thirty grams of fruit tissue (using a corkborer, to remove approximately 10 mm depth plug of the exocarp) were removed and weighed before freezing at −20°C. The tissues were freeze dried (Labconco, TW-9D, Kansas City, MO) and 0.05 g (about 1.2 g fresh weight) was ground into powder using a blender (Newhart Ford, 5010-S, CT) then mixed with 1.0 ml buffer (0.1 M sodium acetate, 1.3 M NaCl and 40 mM 2-mercaptoethanol, pH 6). The slurry was agitated for 1 h at 4°C on a roller-mixer and then centrifuged at 12,500 rpm for 20 min. The crude enzyme extract was immediately assayed.

PG activity was assayed in 2.5 ml substrate stock (prepared by adding 20 ml of 0.6 M NaCl, 80 ml of 75 mM EDTA, and 0.3% polygalacturonic acid [Sigma Chemical, St. Louis, MO], pH 5.0), 0.5 ml of crude enzyme extract, and 0.5 ml of distilled water according to the method of Collmer et al. (1988). The absorbance was measured at 500 nm in a spectrophotometer (Spectronic 601, Milton Roy).

The PG activity was determined using the formula

$$\mathrm{PG} = \frac{\mathrm{Ab_1\text{-}Ab_2}}{\mathrm{Ab_{st}}} \times \frac{S_c \times D_f}{W_s}.$$

Where Ab_1 was the absorbance of substrate mixture; Ab_2 was the absorbance of the control without substrate Ab_{st} was the absorbance of the standard, S_c is the concentration of standard (1.1×10^3 μM), D_f is the dilution factor, and W_s is the weight (0.05 g) of dried sample. The enzyme activity was expressed as PG activity μmol/g dry wt of tissue.

2.5. The effect of hormetic UV-C on PG activity as it relates to fruit firmness

In the first experiment 20 fruits were used in each treatment. Fruit softness (firmness) was measured using a Universal Penetrometer (Humboldt, Co., Chicago 31, IL, USA). The softness was expressed in millimeters according to the procedure described by Bourne (1978). The PG activity in the tissue samples was assayed according to the procedure described above, and this experiment was repeated twice with similar results.

2.6. Effect of hormetic UV-C on tomatoes resistance to PG activity secreted by R. stolonifer infection

In the second experiment two sets of tomatoes were artificially inoculated or not with *R. stolonifer*. The tissue assayed for PG from the latter set (control), was designated as non-decayed tissue. The effect of UV-C treatment on Rhizopus soft rot development and PG activity in decaying tissue of 'Better Boy' tomatoes inoculated with *R. stolonifer* was evaluated. A time course analysis of PG activity in fruit tissue extracts was determined, by removing the lesion using a sterile scalpel. It was then weighed, freeze dried and assayed for PG at 2, 24, 48, 72, and 96 h, after UV-C treatment. This experiment was repeated twice with similar results.

2.7. Statistical analysis

Treatments were replicated four times in a randomized block design with five fruits per replication for a total of 20 fruits per treatment. All data were statistically analyzed using SAS analysis of variance (ANOVA) package. Treatment means were compared by orthogonal comparisons (SAS Institute, 1995).

3. Results and discussion

3.1. The effect of hormetic UV-C on PG activity as it relates to fruit firmness

Results of fruits treated with UV-C at stage 1 of maturity were significantly firmer than non-irradiated

Table 1
Effects of hormetic UV-C exposure (3.6 KJ/m^2) on host polygalacturonase activity and firmness of 'Better Boy' and 'Floradade' tomatoes at different maturity stages

Maturity stages	Polygalacturonase activity ($\times 10^6$ μmol/g dry wt)				Softness of fruits treated at maturity stages (mm)			
	UV-C		Control		UV-C		Control	
	Better boy	Floradade	Better boy	Floradade	Better boy	Floradade	Better boy	Floradade
1	5.19	5.17	6.15	7.93	4.3	ND	6.5	ND
2	5.35	6.35	7.77	10.21	7.5	ND	11.0	ND
3	6.84	10.21	12.45	14.87	10.5	ND	16.0	ND
Average	5.79	7.24	8.79	11.00	7.4		11.2	
Significance of F-test analysis of variance								
Cultivars			NS				NS	
Treatment			**				**	
Maturity			**				**	
Cultivars × maturity			**				**	
Treatment × maturity			**				**	
Cultivars × treatment × maturity			NS				NS	
Treatment × maturity × cultivars			**					

ND = not determined.

control fruits at same stage of maturity (Table 1). Results were similar to those reported by Liu et al. (1993). As firmness increased following UV-C treatment, PG activity decreased. The enzyme activity was lower for UV-C treated fruits than the control (Table 1). The softening of tomato fruits was shown to be closely associated with increases in the activity of pectic enzymes. Fruits of many crops produce a battery of pectic enzymes, i.e. pectate lyase, pectin methyl esterase, ß-galactosade and PG. PG may be most important only very late in soft ripening (Hadfield and Bennett, 1998).

3.2. The effect of hormetic UV-C on tomato resistance to PG secreted by R. stolonifer

The PG activity in crude extract from decayed tomato tissue infected with *R. stolonifer* was higher than PG activity from the extract in non-decayed fruits. UV-C treated tomatoes showed a 40% reduction in PG activity in decayed tissue extract compared to the control at 72 h after UV-C treatment (Table 2). The lesion diameter and percent infection of non-treated and UV-C treated tomatoes at 72 h after treatment, was 13.4 mm (100% infection), and 5.3 mm (47% infection), respectively (Table 2).

3.3. Hormetic UV-C induced resistance of tomatoes to rhizopus soft rot as related to PG suppression in terms of firmness and resistance to degradation of the enzyme

There are numerous biochemical and physiological host defense responses, that may act in concert to explain induced resistance in tomatoes following UV-C treatment (Liu et al., 1993; Charles, 1998; Stevens et al., 1998). The ripening process in tomato fruits involves changes that could influence disease development and increase the susceptibility of fruits and vegetables during ripening: (1) fruits exhibiting advanced maturity associated with a reduction in firmness, resulted in greater susceptibility to storage rots, (2) and increased susceptibility of plant cell wall to attack by macerating pectic enzymes of the pathogen (Eckert, 1978).

Softening of tomato exocarp increased concomitantly with increased PG activity in non-irradiated treated tomatoes. In contrast, UV-C treated fruits were firmer and PG activity was lower (Tables 1 and 2). Previously, it has been reported that the susceptibility of strawberries to attack by *Botrytis cinerea* was highly correlated with the soluble pectin content of the fruit (Hondelmann and Richter, 1973). Recently, Charles (1998), using ultrastructure evaluation with electron microscopy and histochemical studies showed the occurrence of important physical changes, which enhanced the mechanical strength of the cell walls in UV-C treated tomatoes compared to the control. Those physical barriers, prevented or restricted pathogen invasion. Presumably, with a higher pectin content those barriers developed as phytoalexin protection was decreased, thus offering prolonged protection, along with the cell walls which provided resistance to the degradative action of hydrolytic enzymes (i.e. pectolytic enzymes secreted by *B. cinerea*; Charles, 1998).

The results clearly showed that there was suppression of PG activity in tissue extract from decayed lesions infected with *R. stolonifer* of UV-C treated tomatoes compared to the control (Table 2). Pectic enzymes are capable of maceration and killing the cells which are characteristic of diseases caused by plant pathogens (Bateman and Basham, 1976). The evidence of PG activity suppression, suggest that UV-C treatment

Table 2
Effects of hormetic UV-C treatment exposure (3.6 KJ/m^2) on fruit polygalacturonase activity (PG), lesion diameter (LD), and percent infection (%) in *R. stolonifer* artificially inoculated and non-inoculated 'Better Boy' tomatoes

Time after treatment (h)	Polygalacturonase activity ($\times 10^6$ μmol/g dry wt)				Rhizopus soft rot parameter			
	Non-decayed tissue		Decayed tissue		Lesion diameter		Percent infection (cm)	
	UV-C	Control	UV-C	Control	UV-C	Control	UV-C	Control
2	8.77	8.63	49.42	46.25	6.0	6.8	80	100
24	9.90	12.21	52.07	51.09	6.7	11.6	67	100
48	7.66	12.61	46.06	54.37	6.2	12.4	57	100
72	7.26	13.57	36.74	60.10	5.3	13.4	47	100
96	11.10	13.56	53.74	62.00	17.1	14.3	87	100
Significance of F-test from analysis of variance								
			PG		LD		%	
UV-C treatment vs. control			**		**		**	
Time			**		**		**	
Decayed tissue vs. non-decayed tissue			**		NS		NS	
UV-C treatment vs. time			**		NS		NS	
Decayed tissue vs. non-decayed tissue vs. treatment			**		NS		NS	
Decayed tissue vs. non-decayed tissue vs. time			NS		NS		NS	
UV-C treatment vs. time vs. decayed tissue vs. non-decayed tissue			**		NS		NS	

probably reduced the decline of PG inhibiting proteins (IP) during delayed ripening. Normally, the IP decreases with ripening (Albersheim and Anderson, 1971). These inhibitors are typically effective against the PG activity produced by many fungal pathogens (Albersheim and Anderson, 1971). Thus making UV-C treated tomato resistant to PG secreted by *R. stolonifer*. Further research is warranted to test this hypothesis.

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